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Expression of the low-affinity nerve growth factor receptor enhances β-amyloid peptide toxicity

(p75^{NGFR}/TrkA/neurodegenerative disease)

SHAHROOZ RABIZADEH*, CATHERINE M. BITLER†, LARRY L. BUTCHER‡, AND DALE E. BREDESEN§¶

Departments of *Neurology and ‡Psychology, University of California, Los Angeles, CA 90024; †Stanford Research Institute, Menlo Park, CA 94025; and §Department of Neurology, Brain Research Institute, and Molecular Biology Institute, University of California, Los Angeles, CA 90024-1769

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ABSTRACT **The low-affinity nerve growth factor receptor (NGFR) p75^{NGFR} induces apoptosis in the absence of nerve growth factor (NGF) binding but enhances neural survival when bound by NGF. Basal forebrain cholinergic neurons express the highest levels of p75^{NGFR} in the adult human brain and are preferentially involved in Alzheimer disease, raising the question of whether there may be a functional relationship between the expression of p75^{NGFR} and basal forebrain cholinergic neuronal degeneration in Alzheimer disease. The expression of p75^{NGFR} by wild-type and mutant PC12 cells potentiated cell death induced by β-amyloid peptide. NGF binding to p75^{NGFR} inhibited the toxicity of β-amyloid peptide, whereas NGF binding to TrkA, the high-affinity NGFR, enhanced it. These results suggest a possible link between β-amyloid peptide toxicity and preferential degeneration of cells expressing p75^{NGFR}.**

A hallmark of Alzheimer disease is an early and severe telencephalic cholinergic deficit preferentially involving temporal lobe and limbic cortical structures, hippocampus, and amygdala (1–3). The degree of cholinergic decrement correlates well with the severity of dementia (3). Decreases in other neurotransmitters occur to a lesser extent (3), and such deficits are not proportional to the magnitude of intellectual impairment (4).

Virtually all of the cholinergic innervation of the outer cerebral mantle derives from the basal nuclear complex, which is composed of the medial septal nucleus, nuclei of the diagonal band, magnocellular preoptic area, ventral pallidum/substantia innominata region, nucleus basalis, and the nucleus of the ansa lenticularis (5–7). This constellation of cholinergic neurons undergoes degeneration in Alzheimer disease and in at least 13 other diseases in which dementia features prominently (8–10), leading to the question of what characteristic renders these cells selectively vulnerable in those conditions (8).

β-amyloid peptide (βAP) has been shown to be neurotoxic in primary neural cell cultures (11). Moreover, βAP has been implicated in the pathogenesis of Alzheimer disease by the discovery of mutations in the β-amyloid precursor protein gene in a small percentage of familial Alzheimer disease patients (12). In addition, the extent of neuronal loss in the basal forebrain of patients with Alzheimer disease is positively correlated with the degree of β-amyloid accumulation in that region (13).

However, the finding of βAP neurotoxicity does not explain the predisposition of the cholinergic neurons of the basal nuclear complex to degeneration in Alzheimer disease. These neurons express the highest levels of p75^{NGFR}, the low-affinity nerve growth factor receptor (NGFR), in the brain; in contrast, neurons of the other major cholinergic complex in the mammalian brain, the pedunculopontine and laterodorsal tegmental nuclei, neither express p75^{NGFR} nor undergo degeneration in Alzheimer disease (14, 15). p75^{NGFR} expression has been demonstrated to enhance apoptosis in the unbound state, whereas, when p75^{NGFR} is bound by nerve growth factor (NGF) or monoclonal antibody, cell survival is enhanced (16). Thus the effect of p75^{NGFR} on neural cells may be analogous to that of CD40, another member of the tumor necrosis factor receptor superfamily, on centrocytes (17).

Therefore, the effect of p75^{NGFR} expression on βAP neurotoxicity was evaluated. We report that the expression of p75^{NGFR} decreases the median lethal dose of βAP on PC12 cell mutants by approximately one order of magnitude. These results suggest a possible link between βAP toxicity and preferential degeneration of cells expressing p75^{NGFR}.

MATERIALS AND METHODS

Preparation of PC12 Pheochromocytoma Cell Mutants. The derivation of the PC12 cell mutants used in these studies has been described (16). Analysis of the cells for the expression of NGFRs p75^{NGFR} and TrkA, the high-affinity NGFR, included immunoprecipitation, flow cytometry, receptor cross-linking, Northern blots, and immunoblots.

Cell Culture and Expression Constructs. PC12 and PC12-derived mutant cells were maintained and grown as described (18), as were CSM14.1 cells (16). Derivation of the pBabe-puro-p75^{NGFR} and pBabe-puro plasmids and retroviruses was also as described (16).

PC12 NRA5 and CSM14.1 cells were transfected with pBabe-puro and pBabe-puro-p75^{NGFR} by using the cationic lipid *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methyl sulfate (DOTAP, Boehringer Mannheim) according to the supplier protocol. Stably transfected cells were selected in puromycin (10 μg/ml). Pools containing >100 stably transfected colonies were used rather than single colonies, to avoid the bias inherent in comparing single colonies (16). Viability of cells expressing p75^{NGFR} in serum-free medium and βAP (25–50 μM) was determined by trypan blue exclusion, as described (16). The βAP fragment βAP-(1–40), a kind gift from Athena Neurosciences (San Francisco), was dissolved in sterile tissue culture water (Mediatech, Herndon, VA) at a concentration of 1 mM and further diluted in serum-free medium. Preincubation at 37°C for 48 h was carried out prior to use of the peptide, except from one synthesis of βAP, in which preincubation was demonstrated

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Abbreviations: NGF, nerve growth factor; NGFR, NGF receptor; βAP, β-amyloid peptide.

¶To whom reprint requests should be addressed at: Weinberg Laboratory for Neurodegenerative Studies, 710 Westwood Plaza, Los Angeles, CA 90024-1769.

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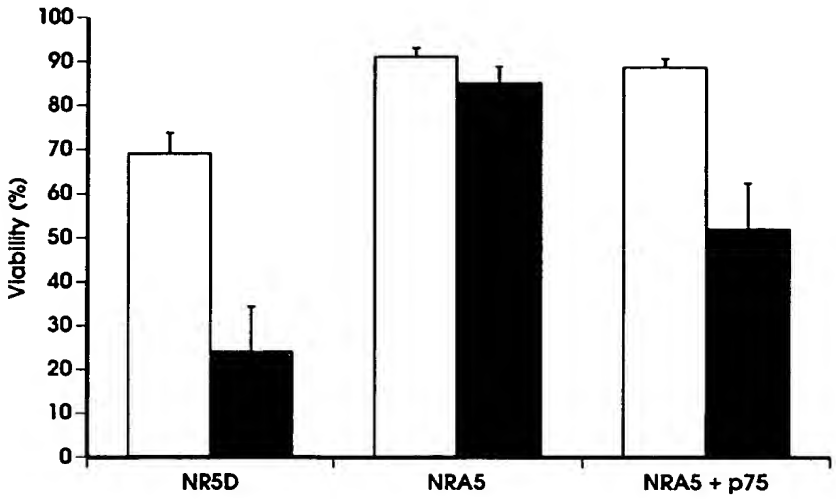

FIG. 1. Viability of PC12 cell mutants exposed to 50 μM βAP for 3 days. The mutant PC12 cells were derived, characterized, and maintained as described (16). NR5D cells express $p75^{NGFR}$, whereas NRA5 cells do not (16). Bars: open, no βAP added; solid, 50 μM βAP added. Loss of viability after exposure to 50 μM βAP for 3 days was highly statistically significant ($P = 0.001$ by two-way analysis of variance; $n = 6$) for the cells expressing $p75^{NGFR}$ (NR5D and NRA5 infected with the pBabe-puro-$p75^{NGFR}$ retrovirus) but was not statistically significant ($P = 0.232$ by two-way analysis of variance; $n = 8$) for the cells not expressing $p75^{NGFR}$ (NRA5). Error bars represent the SEM.

to lead to reduced toxicity on primary neuronal cultures. Results with various preparations of βAP were indistinguishable. Viability assays were performed on cells from four stable transfections, all producing similar results.

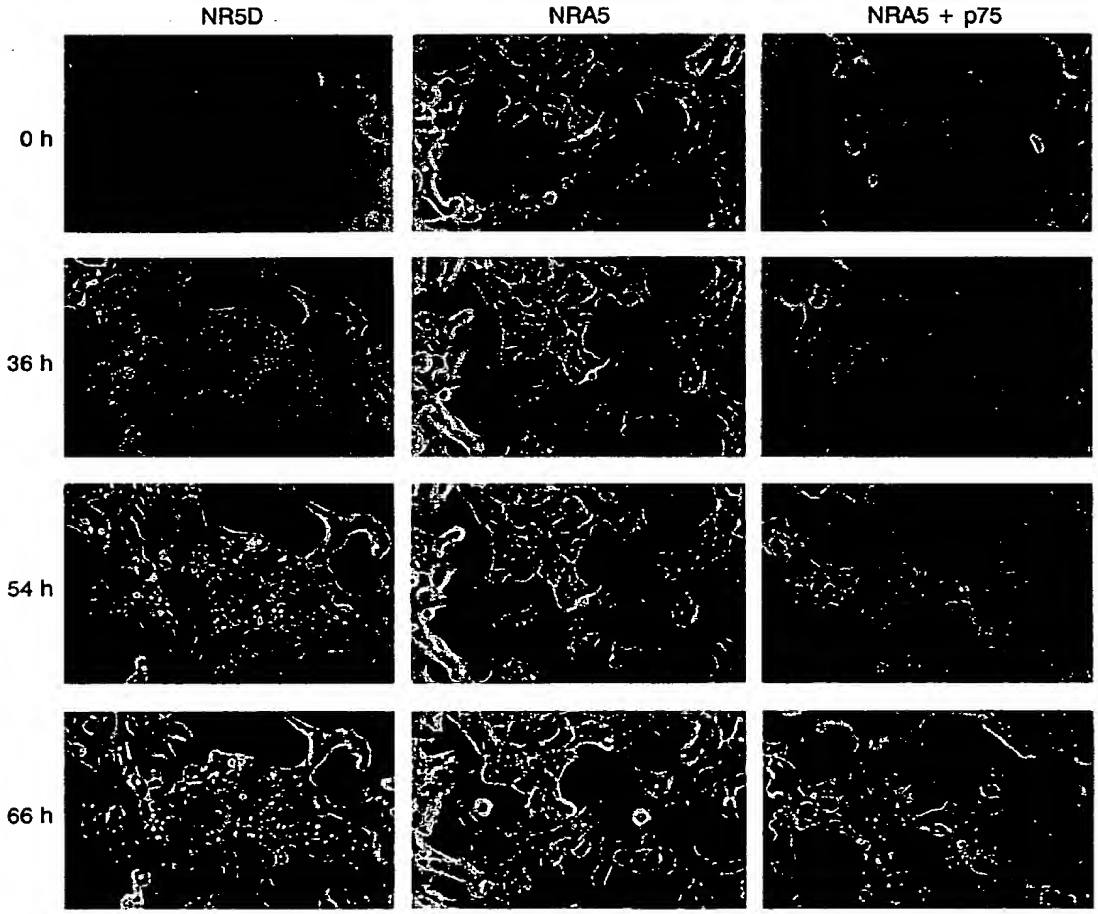

FIG. 2. Photomicrographs of PC12 cell mutants exposed to 50 μM βAP (data presented quantitatively in Fig. 1). (*Left*) NR5D cells exposed to 50 μM βAP for 0, 36, 54, and 66 h (top to bottom, respectively). The same field was photographed each time. Note the development of vacuoles and the overall loss of secondary cellular structure with enlargement of the acellular spaces. (*Center*) NRA5 cells exposed to 50 μM βAP for 0, 36, 54, and 66 h, (top to bottom, respectively). The same field was photographed each time. Note the development of few vacuoles and the retention of overall secondary cellular structure without change in the acellular spaces. (*Right*) NRA5 cells stably infected with the pBabe-puro-$p75^{NGFR}$ retrovirus and exposed to 50 μM βAP for 0, 36, 54, and 66 h (top to bottom, respectively). The same field was photographed each time. Note the marked loss of overall secondary cellular structure, with a marked increase in the acellular spaces. NRA5 cells transfected with pBabe-puro demonstrated resistance to βAP that was statistically not significantly different than NRA5.

Assessment of Protein Expression. After transfection with pBabe-puro or pBabe-puro-p75^{NGFR}, p75^{NGFR} expression in CSM14.1 was assessed by immunocytochemistry with monoclonal antibody 192 as primary antibody, as described (16); expression in PC12 and PC12 mutant cells was determined by flow cytometry using monoclonal antibody 192 (1:100 dilution) as primary antibody and fluorescein-labeled goat anti-mouse IgG + IgM (Kirkegaard & Perry Laboratories) as secondary antibody.

RESULTS

PC12 cell mutants lacking or retaining expression of p75^{NGFR} (16) were assayed for βAP neurotoxicity. Parental PC12 cells are sensitive to the toxicity of βAP (19). NRA5, a mutant of PC12 that lacks p75^{NGFR} expression as demonstrated by immunoprecipitation, immunoblot analysis, flow cytometry, Northern blot analysis, and receptor cross-linking, was much less sensitive to the toxic effect of βAP than NR5D, a mutant of PC12 derived in parallel with NRA5 that expresses p75^{NGFR} (16) (Figs. 1 and 2). Furthermore, when p75^{NGFR} expression was restored to the NRA5 mutant by infection with the pBabe-puro-p75^{NGFR} retrovirus, βAP neurotoxicity was also restored, with a shift of the median lethal dose of βAP of one order of magnitude (Figs. 1 and 2). This did not occur when the NRA5 mutant was infected with the pBabe-puro control retrovirus lacking the p75^{NGFR} coding sequence. A similar enhancement of the neurotoxicity of βAP occurred when another neural cell line, CSM14.1 (16, 20), was transfected with pBabe-puro-p75^{NGFR} (data not shown).

The effect of NGF on the neurotoxicity of βAP was found to be dependent on receptor type: NR6A cells, PC12 mutant cells that express p75^{NGFR} but not TrkA (16), were sensitive to βAP toxicity, with a LD_{50} of ≈5 μM, but the LD_{50} increased to ≈50 μM in the presence of NGF (Fig. 3*A*). Addition of a mutant NGF (K32A, K34A, E35A; at 5 nM) that binds TrkA but not p75^{NGFR} (21) had no effect on βAP toxicity in the NR6A cells, confirming the finding that NGF reduces βAP toxicity in NR6A cells through an interaction other than binding to TrkA (possibly by binding to p75^{NGFR}). In contrast, NRA5 cells, PC12 mutants that express TrkA but not p75^{NGFR}, showed no increase in LD_{50} for βAP when treated with NGF, and in fact, showed a small but statistically significant decrease (Fig. 3*B*).

DISCUSSION

These results suggest that neural cells expressing p75^{NGFR} are more susceptible to the toxic effects of βAP than otherwise similar cells that do not express p75^{NGFR}. The results also provide an example of an effect of NGF that is mediated by p75^{NGFR} but not TrkA and demonstrate that the effect of NGF on βAP neurotoxicity is dependent on which NGFR is bound. This finding and that of Yankner *et al.* (22) showing an increase in βAP toxicity with concentrations of NGF compatible with high-affinity binding suggest that NGF may produce competing cellular survival effects in response to βAP.

It is noteworthy that the cholinergic neurons of the basal forebrain express very high levels of p75^{NGFR} and are affected early and severely in Alzheimer disease; in contrast, morphologically similar cholinergic neurons of the brainstem, which do not express p75^{NGFR}, are not affected (14, 15). Cortical neurons do not express p75^{NGFR} in the normal adult primate brain but do express p75^{NGFR} in temporal association with degeneration, both during development (23) and in Alzheimer disease (24). Furthermore, the administration of βAP to primary cultures of hippocampal neurons induces p75^{NGFR} (22); our results suggest a possible explanation for why these cells may be susceptible to low concentrations of βAP. Purkinje cells also express relatively high levels of p75^{NGFR} (25) but are not prone to degeneration in Alzheimer disease. This may be because the cerebellum does not develop congophilic β-amyloid deposits during the course of Alzheimer disease (26); alternatively, Purkinje cells may differ from neurons of the basal nuclear complex in the expression of another gene or genes that inhibit p75^{NGFR}-mediated neural cell death, as the expression of *bcl-2* does in PC12 cells (18).

Degenerative changes in the cholinergic basal nuclear complex are not limited to Alzheimer disease; other diseases such as Parkinson disease, Pick disease, Creutzfeldt–Jakob disease, subacute sclerosing panencephalitis, progressive supranuclear palsy, dementia pugilistica, olivopontocerebellar atrophy, and the Parkinson-dementia complex of the Chamorro natives of Guam demonstrate neuronal degenerative changes in the nucleus basalis (8, 9). Thus the nucleus basalis has been noted to be a particularly vulnerable nucleus to a wide range of neurodegenerative processes (8). The results presented here and previous results demonstrating

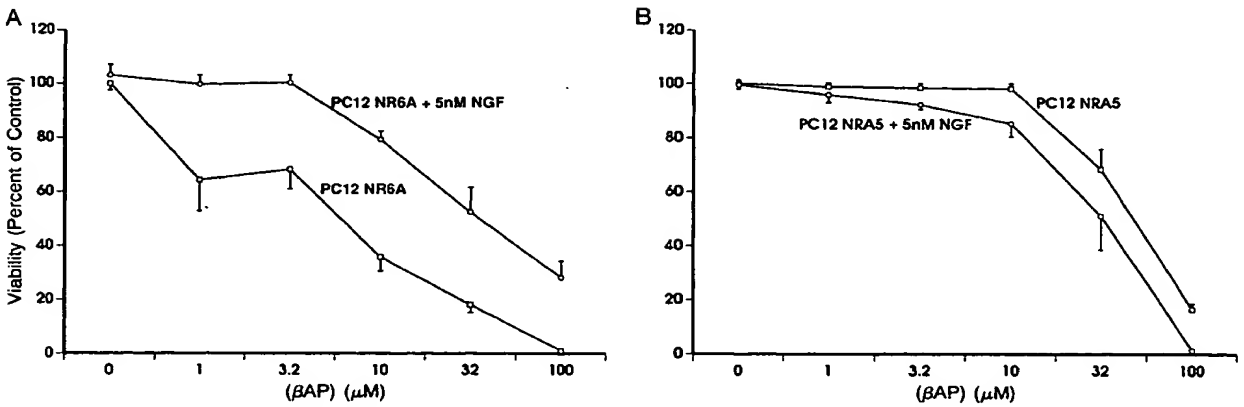

FIG. 3. NGF inhibition of βAP-induced neural cell death is mediated by p75^{NGFR}, whereas NGF enhancement of βAP-induced neural cell death is mediated by TrkA. PC12 mutants were grown and maintained as described (16) and treated with βAP as described in Fig. 1. NR6A cells express p75^{NGFR} but not TrkA, whereas NRA5 cells express TrkA but not p75^{NGFR} (16). (*A*) Effect of NGF (5 nM) on βAP-induced cell death in NR6A cells. NGF led to a highly significant reduction in βAP-induced cell death ($P < 0.001$ by three-way analysis of variance; $n = 3$). (*B*) Effect of NGF (5 nM) on βAP-induced cell death in NRA5 cells. NGF led to a significant augmentation in βAP-induced cell death ($P < 0.05$ by three-way analysis of variance; $n = 3$).

enhancement of neural apoptosis by the expression of $p75^{NGFR}$ (16) suggest the possibility that the vulnerability of basal nuclear neurons and their projections may relate, at least in part, to their high-level expression of $p75^{NGFR}$.

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